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(54) Title: TRIPHENYLMETHANE KINESIN INHIBITORS

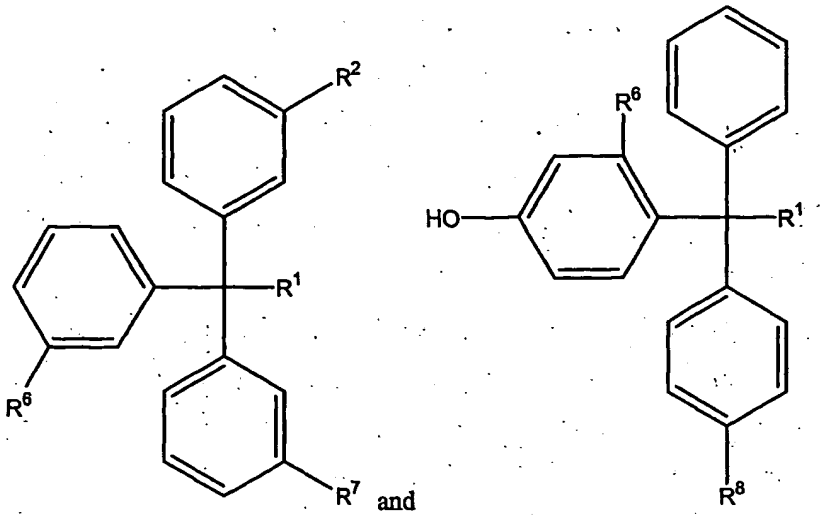
(57) Abstract: Triphenylmethane derivatives of the formula (I) are disclosed. The compounds are inhibitors of the mitotic kinesin KSP and are useful in the treatment of cellular proliferative diseases, such as cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders and inflammation

into mechanical force which drives the directional movement of cellular cargoes along microtubules. The catalytic domain sufficient for this task is a compact structure of approximately 340 amino acids. During mitosis, kinesins organize microtubules into the bipolar structure that is the mitotic spindle. Kinesins mediate movement of chromosomes along spindle microtubules, as well as structural changes in the mitotic spindle associated with specific phases of mitosis. Experimental perturbation of mitotic kinesin function causes malformation or dysfunction of the mitotic spindle, frequently resulting in cell cycle arrest and cell death.

[0006] Among the mitotic kinesins which have been identified is KSP. KSP belongs to an evolutionarily conserved kinesin subfamily of plus end-directed microtubule motors that assemble into bipolar homotetramers consisting of antiparallel homodimers. During mitosis KSP associates with microtubules of the mitotic spindle. Microinjection of antibodies directed against KSP into human cells prevents spindle pole separation during prometaphase, giving rise to monopolar spindles and causing mitotic arrest and induction of programmed cell death. KSP and related kinesins in other, non-human, organisms, bundle antiparallel microtubules and slide them relative to one another, thus forcing the two spindle poles apart. KSP may also mediate in anaphase B spindle elongation and focussing of microtubules at the spindle pole.

[0007] Human KSP (also termed HsEg5) has been described (Blangy, et al., Cell, 83:1159-69 (1995); Whitehead, et al., Arthritis Rheum., 39:1635-42 (1996); Galgio et al., J. Cell Biol., 135:339-414 (1996); Blangy, et al., J Biol. Chem., 272:19418-24 (1997); Blangy, et al., Cell Motil Cytoskeleton, 40:174-82 (1998); Whitehead and Rattner, J. Cell Sci., 111:2551-61 (1998); Kaiser, et al., JBC 274:18925-31 (1999); GenBank accession numbers: X85137, NM004523 and U37426) , and a fragment of the KSP gene (TRIP5) has been described (Lee, et al., Mol Endocrinol., 9:243-54 (1995); GenBank accession number L40372). Xenopus KSP homologs (Eg5), as well as Drosophila KLP61 F/KRP1 30 have been reported.

[0008] Mitotic kinesins are attractive targets for the discovery and development of novel mitotic chemotherapeutics. Accordingly, it is an object of the present invention to provide methods and compositions useful in the inhibition of KSP, a mitotic kinesin.



wherein

R^1 is hydrogen or lower alkyl;

R^2 is chosen from H, -OH, -F, $-NH_2$, and $-NO_2$;

R^3 is chosen from H, -COOH, -O-(alkyl), and -OH;

R^4 is chosen from H, -OH, and -COO(alkyl);

R^5 is chosen from -S-(alkyl), $-NH_2$, $-N(alkyl)_2$, -OH, -O-(alkyl) and SO_2CH_3;

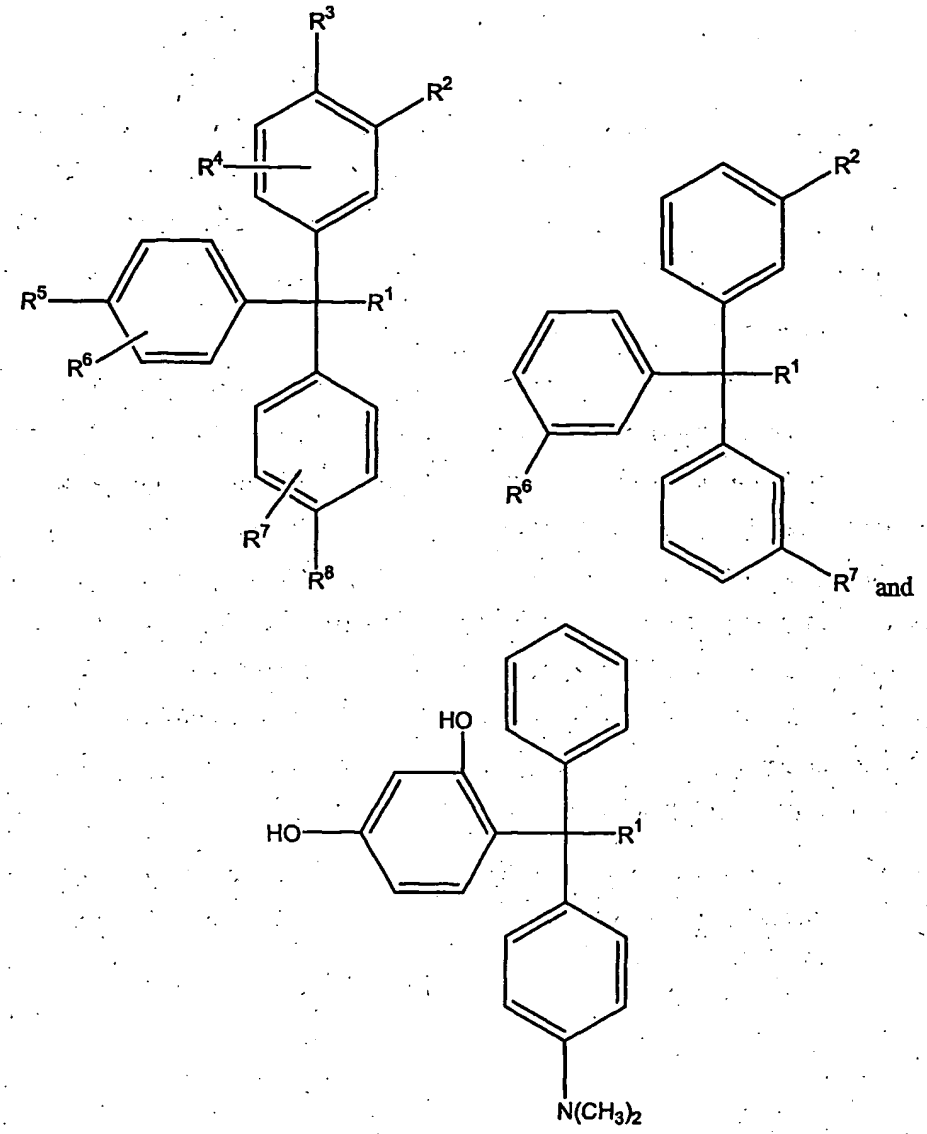
R^6 is chosen from H, $-N(alkyl)_2$, -OH and -COOH;

R^7 is chosen from H, $-N(alkyl)_2$, -OH and -COOH; and

R^8 is chosen from -S-(alkyl), $-NH_2$, $-N(alkyl)_2$, -OH, -O-(alkyl) and SO_2CH_3, wherein at least one of R^2, R^3 and R^4 must be other than hydrogen.

[0012] Diseases and disorders that respond to therapy with compounds of the invention include cancer, hyperplasia, restenosis, cardiac hypertrophy, immune disorders and inflammation.

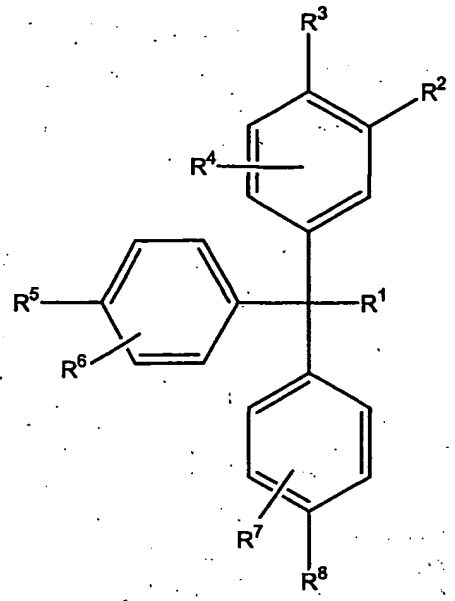
[0013] In another aspect, the invention relates to compounds useful in inhibiting KSP kinesin. The compounds have the structures shown above.



wherein the substituents are as defined before.

DETAILED DESCRIPTION OF THE INVENTION

[0017] The present invention is directed to a class of novel triphenylmethanes that are modulators of mitotic kinesins. By inhibiting or modulating mitotic kinesins, but not other kinesins (e.g., transport kinesins), specific inhibition of cellular proliferation is accomplished. Thus, the present invention capitalizes on the finding that perturbation of



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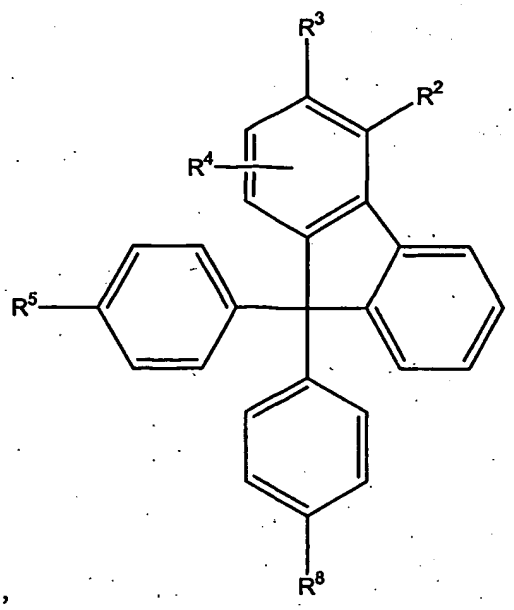
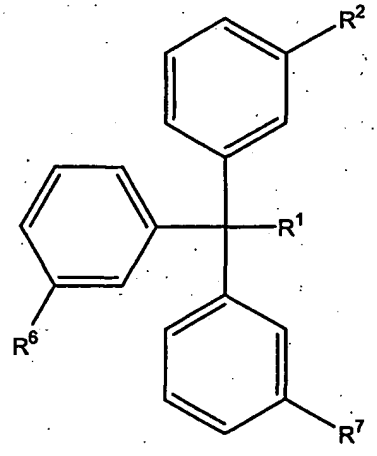
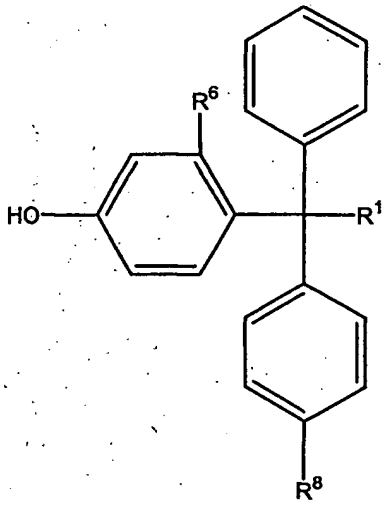




and



[0020] All of the compounds falling within the foregoing parent genus and its subgenera are useful as kinesin inhibitors, but not all the compounds are novel. In particular, certain known species fall within the genus, although no utility in inhibiting kinesin has been suggested for these species. Any narrowing of the claims or specific exceptions that might be added to these claims reflect applicants' intent to avoid claiming

COOH or -OCH_3; (3) R^5 and R^8 are chosen from -N(alkyl)$_2$ and -OH; (4) R^2 is chosen from -OH, -F, and -NH_2; (5) R^6 and R^7 are hydrogen or R^6 and R^7 are -N(alkyl)$_2$; and R^5 and R^8 are chosen from -S-CH_3, -N(lower-alkyl)$_2$ and SO_2CH_3.

Definitions

[0022] Alkyl is intended to include linear, branched, or cyclic hydrocarbon structures and combinations thereof having 12 or fewer carbons. Lower alkyl refers to alkyl groups of from 1 to 5 carbon atoms. Examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s-and t-butyl and the like. Cycloalkyl is a subset of alkyl and includes cyclic hydrocarbon groups of from 3 to 12 carbon atoms. Examples of cycloalkyl groups include c-propyl, c-butyl, c-pentyl, norbornyl, adamantyl and the like. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed; thus, for example, "butyl" is meant to include n-butyl, sec-butyl, isobutyl and t-butyl; "propyl" includes n-propyl and isopropyl.

[0023] Alkoxy or alkoxyl refers to groups of from 1 to 8 carbon atoms of a straight, branched, cyclic configuration and combinations thereof attached to the parent structure through an oxygen. Examples include methoxy, ethoxy, propoxy, isopropoxy, cyclopropyloxy, cyclohexyloxy and the like. Lower-alkoxy refers to groups containing one to four carbons, and such are preferred.

[0024] Halogen refers to fluorine, chlorine, bromine or iodine. Fluorine, chlorine and bromine are preferred.

[0025] Some of the compounds described herein contain one or more asymmetric centers (e.g. the methine carbon when each of the phenyl rings is differently substituted) and may thus give rise to enantiomers, diastereomers (e.g. when R^1 contains a stereogenic center), and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)-. The present invention is meant to include all such possible isomers, including racemic mixtures, optically pure forms and intermediate mixtures. Optically active (R)- and (S)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry,

modulate means either increasing or decreasing spindle pole separation, causing malformation, i.e., splaying, of mitotic spindle poles, or otherwise causing morphological perturbation of the mitotic spindle. Also included within the definition of KSP for these purposes are variants and/or fragments of KSP. See U.S. Patent Application "Methods of Screening for Modulators of Cell Proliferation and Methods of Diagnosing Cell Proliferation States", filed Oct. 27, 1999 (U.S. Serial Number 09/428,156), hereby incorporated by reference in its entirety. In addition, other mitotic kinesins may be used in the present invention. However, the compositions of the invention have been shown to have specificity for KSP.

[0030] For assay of activity, generally either KSP or a compound according to the invention is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g., a microtiter plate, an array, etc.). The insoluble support may be made of any composition to which the compositions can be bound, is readily separated from soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any convenient shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose, Teflon™, etc. Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the composition is not crucial so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the composition and is nondiffusable. Preferred methods of binding include the use of antibodies (which do not sterically block either the ligand binding site or activation sequence when the protein is bound to the support), direct binding to "sticky" or ionic supports, chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following binding of the protein or agent, excess unbound material is removed by washing. The sample receiving areas may then be blocked through incubation with bovine serum albumin (BSA), casein or other innocuous protein or other moiety.

[0031] The antimitotic agents of the invention may be used on their own to modulate the activity of a mitotic kinesin, particularly KSP. In this embodiment, the mitotic agents of the invention are combined with KSP and the activity of KSP is

embodiment, the effect of the agents on kinesin ATPase can be decreased by increasing the concentrations of ATP, microtubules or both. In yet another embodiment, the effect of the modulating agent is increased by increasing concentrations of ATP, microtubules or both.

[0035] Agents that modulate the biochemical activity of KSP in vitro may then be screened in vivo. Methods for such agents in vivo include assays of cell cycle distribution, cell viability, or the presence, morphology, activity, distribution, or amount of mitotic spindles. Methods for monitoring cell cycle distribution of a cell population, for example, by flow cytometry, are well known to those skilled in the art, as are methods for determining cell viability. See for example, U.S. Patent Application "Methods of Screening for Modulators of Cell Proliferation and Methods of Diagnosing Cell Proliferation States," filed Oct. 22, 1999, serial number 09/428,156, hereby incorporated by reference in its entirety.

[0036] In addition to the assays described above, microscopic methods for monitoring spindle formation and malformation are well known to those of skill in the art (see, e.g., Whitehead and Rattner (1998), J. Cell Sci. 111:2551-61; Galgio et al, (1996) J. Cell biol., 135:399-414).

[0037] The compositions of the invention inhibit the KSP kinesin. One measure of inhibition is IC_{50}, defined as the concentration of the composition at which the activity of KSP is decreased by fifty percent. Preferred compositions have IC_{50}'s of less than about 1 mM, with preferred embodiments having IC_{50}'s of less than about 100 μM, with more preferred embodiments having IC_{50}'s of less than about 10 μM, with particularly preferred embodiments having IC_{50}'s of less than about 1 μM, and especially preferred embodiments having IC_{50}'s of less than about 500 nM. Measurement of IC_{50} is done using an ATPase assay.

[0038] Another measure of inhibition is K_i. For compounds with IC_{50}'s less than 1 μM, the K_i or K_d is defined as the dissociation rate constant for the interaction of the triphenylmethane with KSP. Preferred compounds have K_i's of less than about 100 μM, with preferred embodiments having K_i's of less than about 10 μM, and particularly preferred embodiments having K_i's of less than about 1 μM and especially preferred embodiments having K_i's of less than about 500 nM.

glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma); Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor (nephroblastoma), lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma (pinealoma), glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma (serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma), granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes (carcinoma); Hematologic: blood (myeloid leukemia (acute and chronic), acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma (malignant lymphoma); Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma. Thus, the term

maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[0046] The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents. The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations.

[0047] The administration of the mitotic agents of the present invention can be done in a variety of ways as discussed above, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in the treatment of wounds and inflammation, the anti-mitotic agents may be directly applied as a solution or spray.

[0048] To employ the compounds of the invention in a method of screening for compounds that bind to KSP kinesin, the KSP is bound to a support, and a compound of the invention (which is a mitotic agent) is added to the assay. Alternatively, the

oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactivity. They may be capable of directly or indirectly altering the cellular proliferation phenotype or the expression of a cellular proliferation sequence, including both nucleic acid sequences and protein sequences. In other cases, alteration of cellular proliferation protein binding and/or activity is screened. Screens of this sort may be performed either in the presence or absence of microtubules. In the case where protein binding or activity is screened, preferred embodiments exclude molecules already known to bind to that particular protein, for example, polymer structures such as microtubules, and energy sources such as ATP. Preferred embodiments of assays herein include candidate agents which do not bind the cellular proliferation protein in its endogenous native state termed herein as "exogenous" agents. In another preferred embodiment, exogenous agents further exclude antibodies to KSP.

[0053] Candidate agents can encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding and lipophilic binding, and typically include at least an amine, carbonyl, hydroxyl, ether, or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Particularly preferred are peptides.

[0054] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or

[0060] In an alternative embodiment, the candidate agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate the candidate agent is bound to KSP with a higher affinity. Thus, if the candidate agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate the candidate agent is capable of binding to KSP.

[0061] It may be of value to identify the binding site of KSP. This can be done in a variety of ways. In one embodiment, once KSP has been identified as binding to the mitotic agent, KSP is fragmented or modified and the assays repeated to identify the necessary components for binding.

[0062] Modulation is tested by screening for candidate agents capable of modulating the activity of KSP comprising the steps of combining a candidate agent with KSP, as above, and determining an alteration in the biological activity of KSP. Thus, in this embodiment, the candidate agent should both bind to KSP (although this may not be necessary), and alter its biological or biochemical activity as defined herein. The methods include both in vitro screening methods and in vivo screening of cells for alterations in cell cycle distribution, cell viability, or for the presence, morpohology, activity, distribution, or amount of mitotic spindles, as are generally outlined above.

[0063] Alternatively, differential screening may be used to identify drug candidates that bind to the native KSP, but cannot bind to modified KSP.

[0064] Positive controls and negative controls may be used in the assays. Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent determined. For example, where a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of bound compound.

[0065] A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be

Et = ethyl

Fmoc = 9-fluorenylmethoxycarbonyl

GC = gas chromatography

HATU = O-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HMDS = hexamethyldisilazane

HOAc = acetic acid

HOBt = hydroxybenzotriazole

Me = methyl

mesyl = methanesulfonyl

MOM = methoxymethyl

MTBE = methyl t-butyl ether

PEG = polyethylene glycol

Ph = phenyl

PhOH = phenol

PfP = pentafluorophenol

PPTS = pyridinium p-toluenesulfonate

Py = pyridine

PyBroP = bromo-tris-pyrrolidino-phosphonium hexafluorophosphate

rt = room temperature

sat=d = saturated

s- = secondary

t- = tertiary

TBDMS = t-butyldimethylsilyl

TES = triethylsilane

TFA = trifluoroacetic acid

THF = tetrahydrofuran

TMOF = trimethyl orthoformate

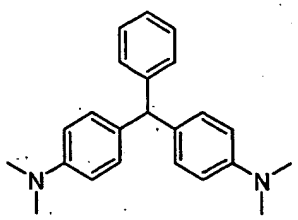
TMS = trimethylsilyl

tosyl = p-toluenesulfonyl

Trt = triphenylmethyl

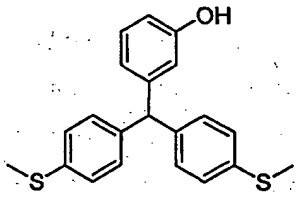
Synthesis of Compounds

Triphenylmethane Procedure A:



[0069] Dimethylaniline (4 mmol) was combined with benzaldehyde (2 mmol) in water (2 mL) and sulfuric acid (100 L) and heated at 95° C for 48 h. The cooled mixture was washed with DCM (2 x 4ml), neutralized with Na_2CO_3, extracted with DCM (2 x 4 mLl), dried ($MgSO_4$) and evaporated affording product (about 1.50 mmol, 75%)

Triphenylmethane Procedure B:



[0070] Ethyl-3-hydroxy benzoate (7.50 g, 45.2 mmol) was dissolved in anhydrous THF (50 ml) and cooled to 0° C. A 60 % aqueous solution of sodium hydride (1.81g, 45.18 mmol) was added in small portions and the mixture was stirred for 10 min. Chloromethylmethyl ether (4.29 ml, 56.5 mmol) was added over 5 min and the mixture was allowed to warm to RT over 1h. The mixture was combined with DCM(150mL) and washed with water (3x 150mL), dried (MgSO4) and evaporated affording a clear oil (9.62g, 101%)

[0071] 4-Bromothioanisole (812 mg, 4.00 mmol) was dissolved in THF (4 mL) and cooled to –78 °C. 1.6M n-butyl lithium (2.50 mL, 4.00 mmol) was added and the mixture was stirred at –78 °C for 30 min. In a second flask, ethylmethoxymethoxy

Monopolar Spindle Formation following Application of a Triphenylmethane KSP Inhibitor

[0075] To determine the nature of the G2/M accumulation, human tumor cell lines Skov-3 (ovarian), HeLa (cervical), and A549 (lung) were plated in 96-well plates at densities of 4,000 cells per well (SKOV-3 & HeLa) or 8,000 cells per well (A549), allowed to adhere for 24 hours, and treated with various concentrations of the triphenylmethane compounds for 24 hours. Cells were fixed in 4% formaldehyde and stained with antitubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which stains DNA).

[0076] Visual inspection revealed that the triphenylmethane compounds caused cell cycle arrest in the prometaphase stage of mitosis. DNA was condensed and spindle formation had initiated, but arrested cells uniformly displayed monopolar spindles, indicating that there was an inhibition of spindle pole body separation. Microinjection of anti-KSP antibodies also causes mitotic arrest with arrested cells displaying monopolar spindles.

Inhibition of Cellular Proliferation in Tumor Cell Lines Treated with Triphenylmethane KSP Inhibitors.

[0077] Cells were plated in 96-well plates at densities from 1000-2500 cells/well of a 96-well plate (depending on the cell line) and allowed to adhere/grow for 24 hours. They were then treated with various concentrations of drug for 48 hours. The time at which compounds are added is considered T_0. A tetrazolium-based assay using the reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (I.S> Patent No. 5,185,450) (see Promega product catalog #G3580, CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay) was used to determine the number of viable cells at T_0 and the number of cells remaining after 48 hours compound exposure. The number of cells remaining after 48 hours was compared to the number of viable cells at the time of drug addition, allowing for calculation of growth inhibition.

[0078] The growth over 48 hours of cells in control wells that had been treated with vehicle only (0.25% DMSO) is considered 100% growth and the growth of cells in

Costar 3695) using Solution 1. Following serial dilution each well has 50 µl of Solution 1. The reaction is started by adding 50 µl of solution 2 to each well. This may be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC_{50} determination the data acquired is fit by the following four parameter equation using a nonlinear fitting program (e.g., Grafit 4):

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^{s}} + \text{Background}$$

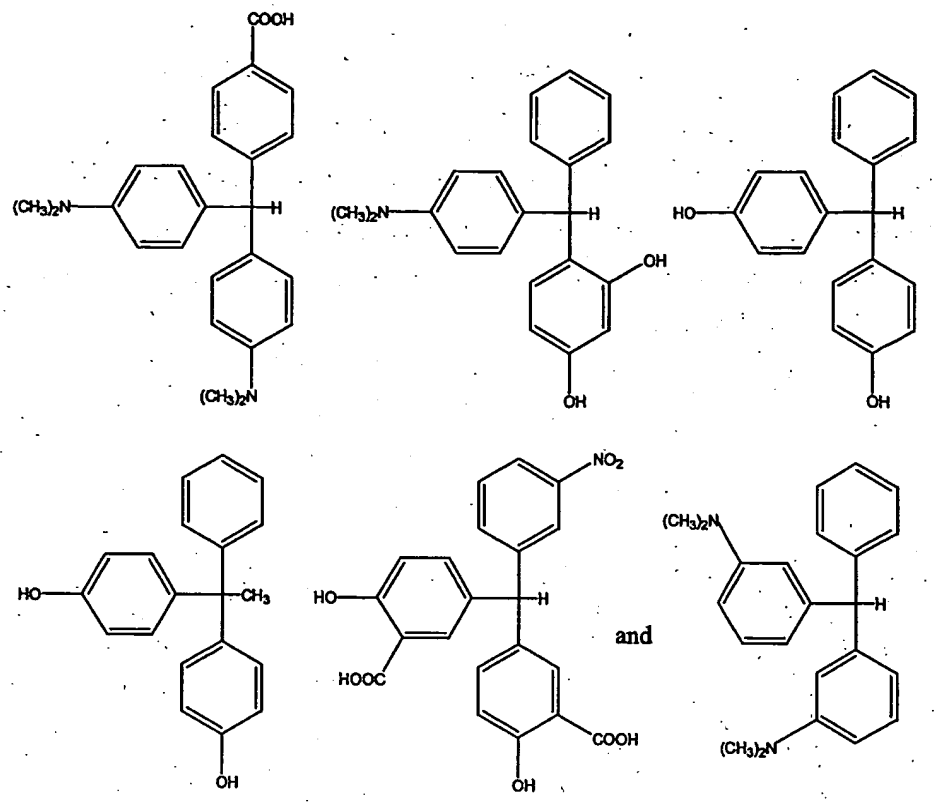
where y is the observed rate and x the compound concentration.

[0082] The K_i for a compound is determined from the IC_{50} based on three assumptions. First, only one compound molecule binds to the enzyme and there is no cooperativity. Second, the concentrations of active enzyme and the compound tested are known (i.e., there are no significant amounts of impurities or inactive forms in the preparations). Third, the enzymatic rate of the enzyme-inhibitor complex is zero. The rate (i.e., compound concentration) data are fitted to the equation:

$$V = V_{max} E_0 \left[I - \frac{(E_0 + I_0 + Kd) - \sqrt{(E_0 + I_0 + Kd)^2 - 4 E_0 I_0}}{2E_0} \right]$$

where V is the observed rate, V_{max} is the rate of the free enzyme, I_0 is the inhibitor concentration, E_0 is the enzyme concentration, and K_d is the dissociation constant of the enzyme-inhibitor complex.

[0083] Several representative compounds of the invention were tested as described above and found to exhibit Ki's below 100 µM. Their structures are as shown:

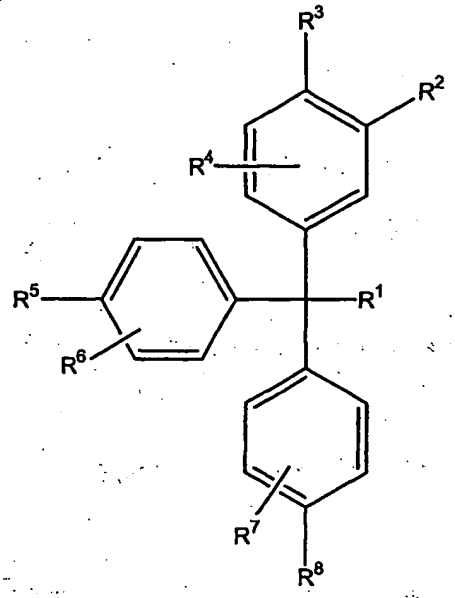


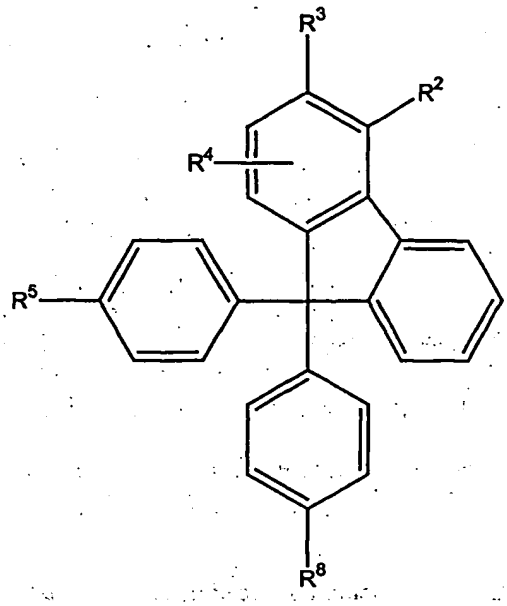
[0084] The triphenylmethane compounds inhibit growth in a variety of cell lines, including cell lines (MCF-7/ADR-RES, HCT1 5) that express P-glycoprotein (also known as Multi-drug Resistance, or MDR^+), which conveys resistance to other chemotherapeutic drugs, such as pacilitaxel. Therefore, the triphenylmethanes are anti-mitotics that inhibit cell proliferation, and are not subject to resistance by overexpression of MDR^+ by drug-resistant tumor lines.

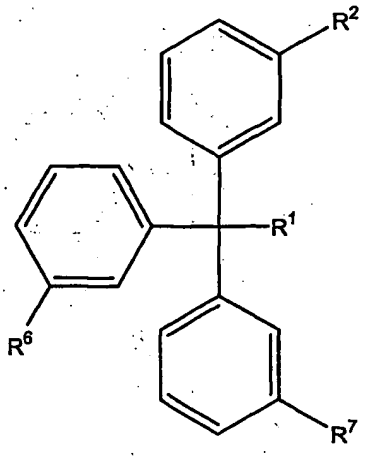
[0085] Compounds of this class were found to inhibit cell proliferation, although GI_{50} values varied. GI_{50} values for the triphenylmethane compounds tested ranged from 200 nM to greater than the highest concentration tested. By this we mean that although most of the compounds that inhibited KSP activity biochemically did inhibit cell proliferation, for some, at the highest concentration tested (generally about 20 μM), cell growth was inhibited less than 50%. Many of the compounds have GI_{50} values less than 10 μM, and several have GI_{50} values less than 1 μM. Anti-proliferative compounds that have been successfully applied in the clinic to treatment of cancer (cancer chemotherapeutics) have GI_{50}'s that vary greatly. For example, in A549 cells, paclitaxel GI_{50} is 4 nM, doxorubicin is 63 nM, 5-fluorouracil is 1 μM, and hydroxyurea is 500 μM

We claim:

1. A method of treating cellular proliferative diseases comprising administering a compound chosen from:







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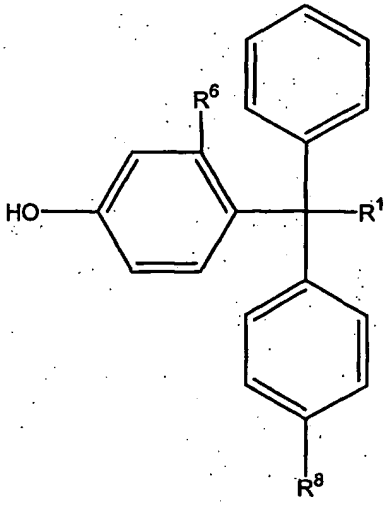
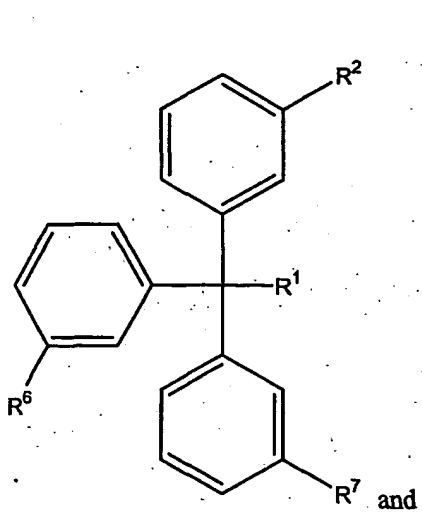
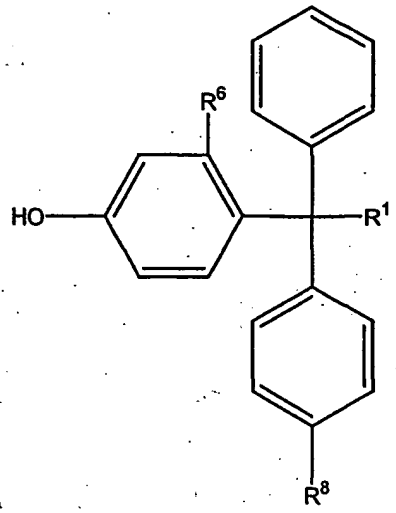






and

wherein

R^1 is hydrogen or lower alkyl;

R^2 is chosen from H, -OH, -F, $-NH_2$, and $-NO_2$;

R^3 is chosen from H, -COOH, -O-(alkyl), and -OH;

R^4 is chosen from H, -OH, and -COO(alkyl);

R^5 is chosen from -S-(alkyl), $-NH_2$, $-N(alkyl)_2$, -OH, -O-(alkyl) and SO_2CH_3;

R^6 is chosen from H, $-N(alkyl)_2$, -OH and -COOH;

R^7 is chosen from H, $-N(alkyl)_2$, -OH and -COOH;

R^8 is chosen from -S-(alkyl), $-NH_2$, $-N(alkyl)_2$, -OH, -O-(alkyl) and SO_2CH_3;

or a pharmaceutically acceptable salt thereof, with the proviso that at least one of R^2, R^3 and R^4 must be other than hydrogen.

3. A method of inhibiting KSP kinesin comprising contacting KSP kinesin with a compound chosen from:

R^5 is chosen from -S-(alkyl), $-NH_2$, $-N(alkyl)_2$, -OH, -O-(alkyl) and SO_2CH_3;

R^6 is chosen from H, $-N(alkyl)_2$, -OH and -COOH;

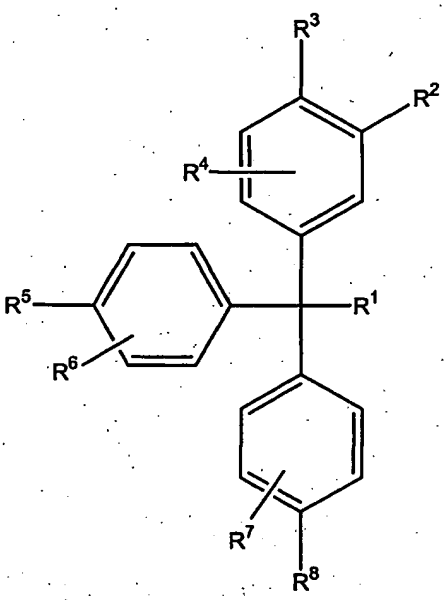
R^7 is chosen from H, $-N(alkyl)_2$, -OH and -COOH;

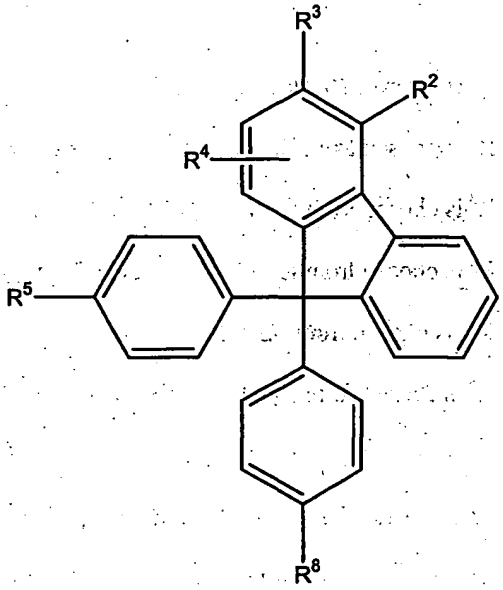
R^8 is chosen from -S-(alkyl), $-NH_2$, $-N(alkyl)_2$, -OH, -O-(alkyl) and SO_2CH_3;

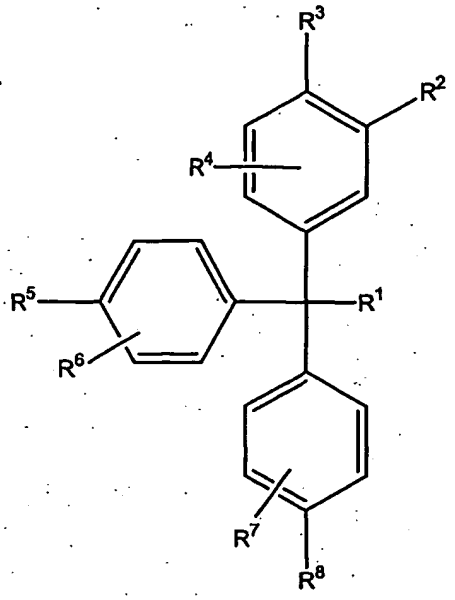
or a pharmaceutically acceptable salt thereof, with the proviso that at least one of R^2, R^3 and R^4 must be other than hydrogen.

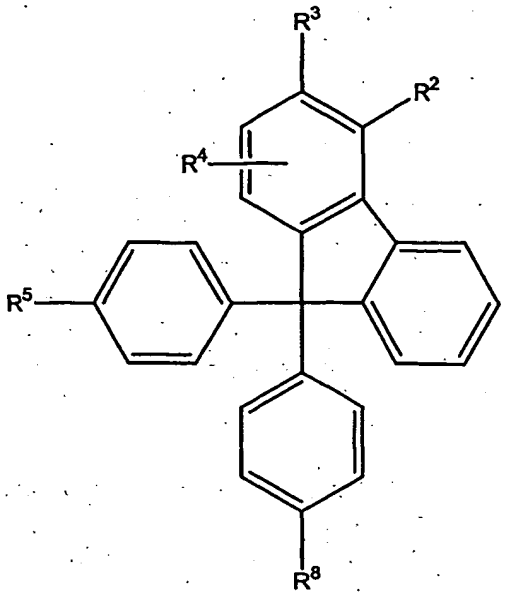
4. A method of screening for KSP kinesin modulators comprising:

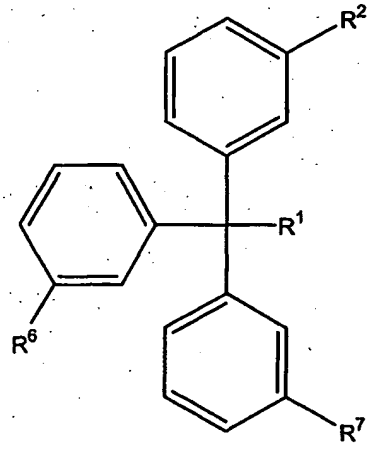
combining a kinesin, a candidate bioactive agent and a compound chosen from:











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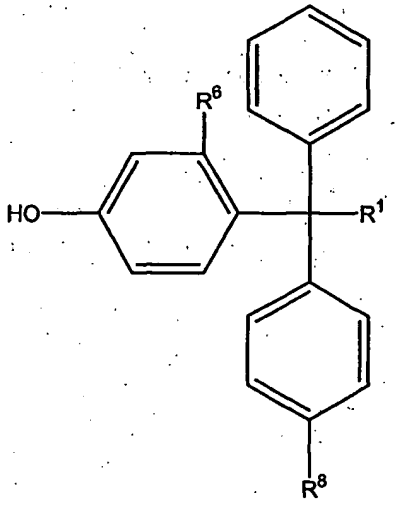


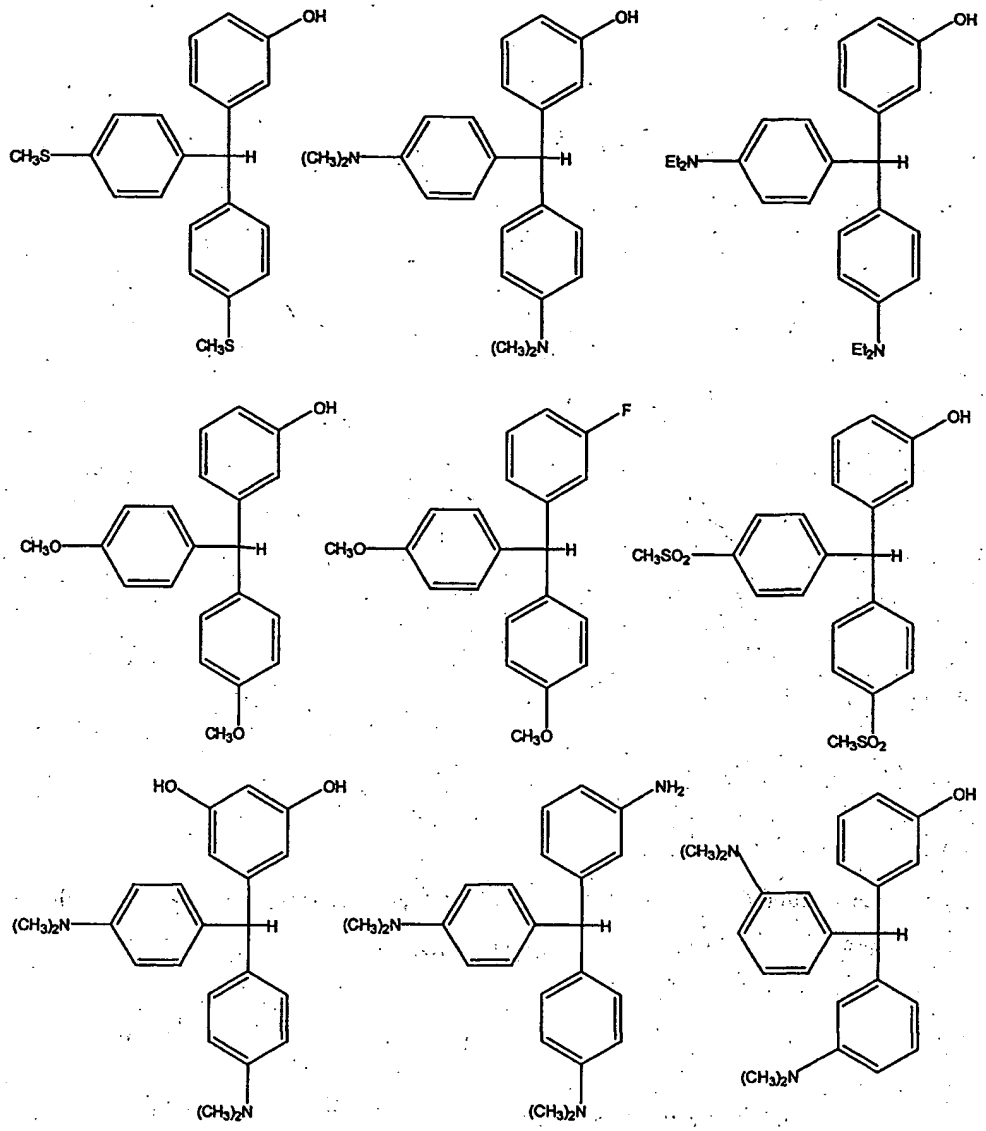
wherein

R^1 is hydrogen or lower alkyl;

R^2 is chosen from H, -OH, -F, $-NH_2$, and $-NO_2$;

R^3 is chosen from H, -COOH, -O-(alkyl), and -OH;

R^4 is chosen from H, -OH, and -COO(alkyl);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CH3S
OH
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H
(CH3)2N
OH
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H
Et2N
OH
CH3O
H
CH3O
F
CH3O
H
CH3O
OH
CH3SO2
H
CH3SO2
HO
OH
(CH3)2N
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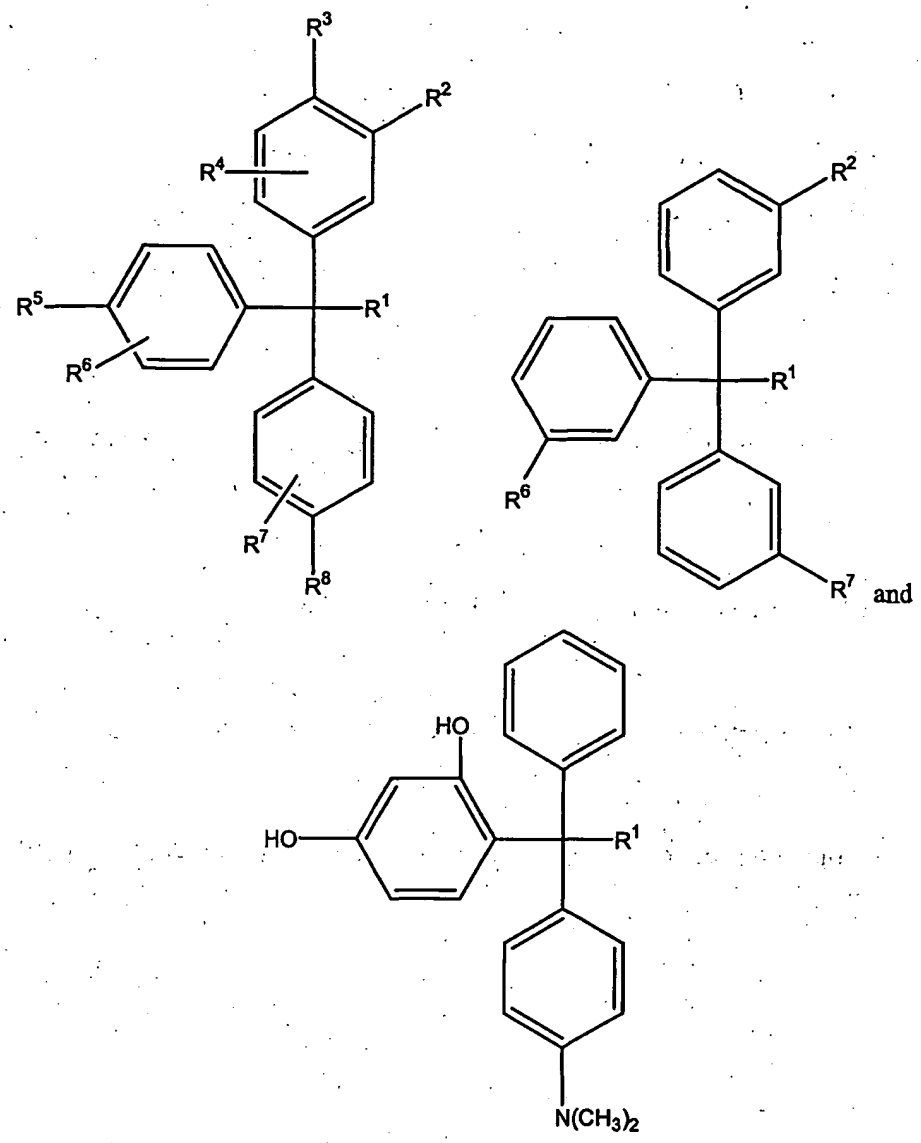

wherein

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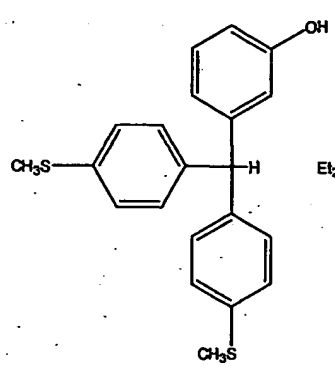
R^2 is chosen from H, -OH, -F, $-NH_2$, and $-NO_2$;

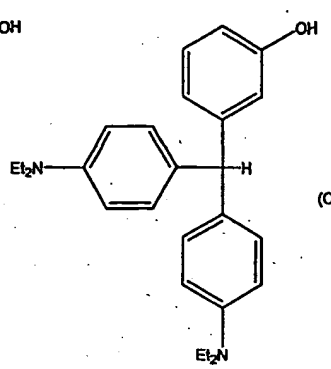
R^3 is chosen from H, -COOH, -O-(alkyl), and -OH;

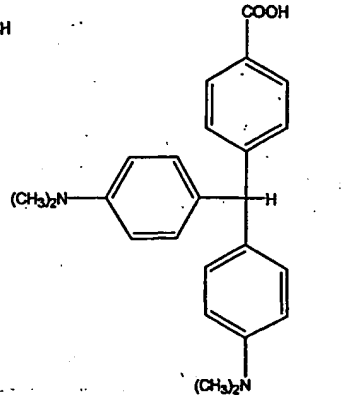
R^4 is chosen from H, -OH, and -COO(alkyl);

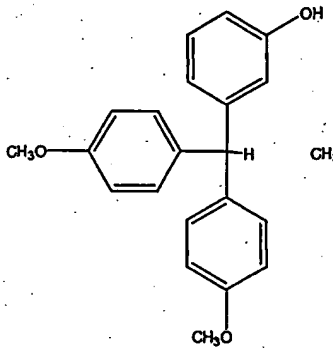
R^5 is chosen from -S-(alkyl), $-NH_2$, $-N(alkyl)_2$, -OH, -O-(alkyl) and SO_2CH_3;

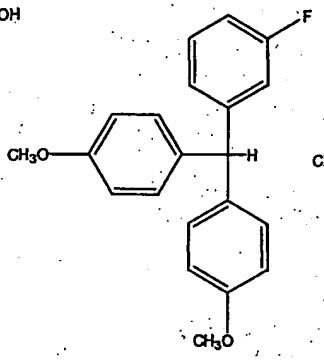
16. A triphenylmethane according to claim 11 chosen from

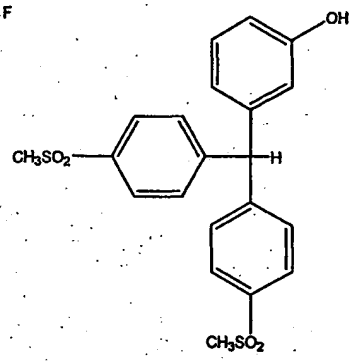


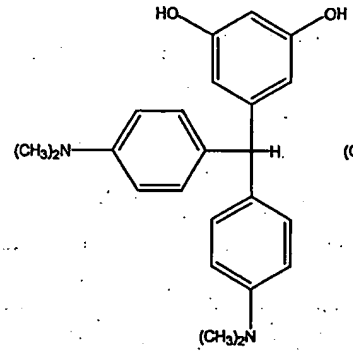


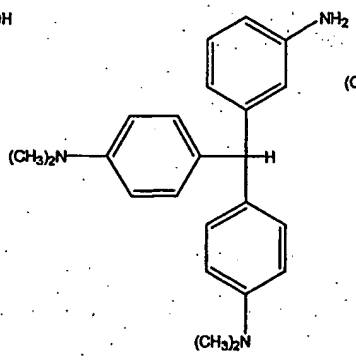


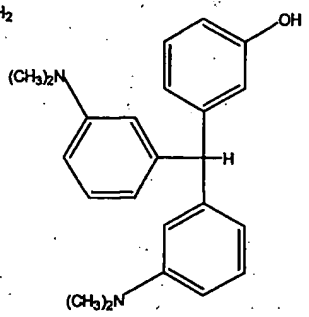


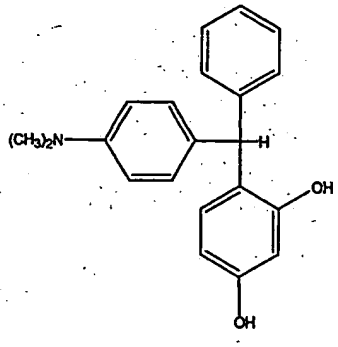


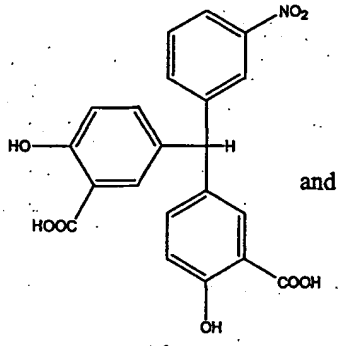












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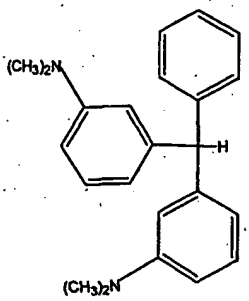

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/01614

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 06137 A (ABBOTT LAB) 10 February 2000 (2000-02-10) abstract page 3, line 13 -page 12, line 25 claims 1-19	11-16
X	EP 1 000 925 A (SUMITOMO CHEMICAL CO) 17 May 2000 (2000-05-17) page 2, line 26 -page 4, line 37 claims 1-7	11-16
X	US 6 166 013 A (COGHLAN MICHAEL J ET AL) 26 December 2000 (2000-12-26) abstract claims 1-9	11-16
P,A	WO 01 31335 A (BERAUD CHRISTOPHE ;FINER JEFFREY T (US); MAK JOHN (US); SAKOWICZ R) 3 May 2001 (2001-05-03) abstract claims 1-59	4-10
A	MUTHYALA, R; KATRITZKY, A R; LAN, X: "A Synthetic Study on the Preparation of Triarylmethanes" DYES & PIGMENTS, vol. 25, 1994, pages 303-324, XP001078895 abstract	11-16